ELECTRONIC SUPPLEMENTARY INFORMATION

Dynamic behavior analysis of ion transport through a bilayer lipid membrane by an electrochemical method combined with fluorometry

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Extraction procedure of rhodamine 6G, $R6G^+$, and BF_4^- with liposomes

The extraction of $R6G^+$ with BF_4^- based on the dialysis membrane method[1–3] was carried out as described in previous work[4]. The aqueous solution was separated using a dialysis tube of regenerated cellulose (diameter of 16 mm, thickness of 20.3 μm, pore size of 5 nm, molecular weight cut-off of 14,000 Da; UC 20-32-100, Viskase Companies Inc., Illinois, USA). The dialysis tube including the aqueous inner solution (1 cm^{-3}), whose top and bottom were tightly tied with Nylon line (Nasuly N-Walker Nylon W-DMV, YGK Yoz-Ami Co., Ltd., Naruto, Japan) to avoid inner solution leakage, was soaked in a test glass tube (height of 180 mm, internal diameter of 15 mm) filled with the outer solution (5 cm^{-3}). The inner solution contained 0.10 M phosphate buffer (pH 7), various concentrations of

$NaBF_4$, and 1.0×10^{-6} mol dm^{-3} R6GCl; whereas the outer solution contained 0.10 mol dm^{-3} phosphate buffer (pH 7), various concentrations of $NaBF_4$, and liposomes consisting of 3.3×10^{-3} mol dm^{-3} PC and 3.3×10^{-3} mol dm^{-3} cholesterol. The liposomes were prepared as described in previous work[4], the size of the liposomes was 140 ± 60 nm. The ionic strength of the aqueous solution was mainly determined by 0.10 mol dm^{-3} phosphate buffer, and all extraction experiments were carried out under the same ionic strength. The test glass tubes with the outer and inner solutions were shaken for 15 h at 20°C in a reciprocal shaker (Taiyo Incubator Personal, Taiyo Kagakukogyo Co., Tokyo, Japan). We confirmed that the extraction time of 15 h was enough to attain extraction equilibrium by measuring the $R6G^+$ concentrations in the inner and outer solutions, where their concentration after extraction indicates the same value. To avoid $R6G^+$ adsorption, the dialysis tube and the glass tube required pretreatment[4].

For the extraction, two sets of test glass tubes were prepared for each experiment: one in the presence of liposomes (the measurement cell) and the other in their absence (the reference cell). The amount of extracted $R6G^+$ was estimated from the difference between the $R6G^+$ concentration in the inner solution of the measurement cell, $[R6G^+]_{mea}$, and that of the reference cell, $[R6G^+]_{ref}$. The concentration of $R6G^+$ in the solution was determined by fluorescence spectrometry (FP6200, Jasco Co., Tokyo, Japan).

Determination of apparent distribution ratio

The apparent distribution ratio, R, of $R6G^+$ between the aqueous phase, W, and the liposome membrane, lip, was defined as the ratio of the concentration of $R6G^+$ in lip ($[R6G^+]_{lip}^T$) to the concentration of $R6G^+$ in W, $[R6G^+]_W$.

$$R = \frac{[R6G^+]_{lip}^T}{[R6G^+]_W} \tag{S1}$$

Here, $[A]_B$ indicates molar concentration of A in phase B. We assumed the ion-pair formation in W to be negligible and the concentration of $R6G^+$ in W to be equal to $[R6G^+]_W$, which was experimentally estimated as $[R6G^+]_{mea}$. $[R6G^+]_{lip}^T$ was estimated as the apparent concentration of $R6G^+$ in lip based on the decrease in $R6G^+$ concentration of the inner solution caused by the addition of the liposome ($[R6G^+]_{ref} - [R6G^+]_{mea}$).

$$[R6G^+]_{lip}^T = \left([R6G^+]_{ref} - [R6G^+]_{mea}\right)\frac{(V_{out} + V_{in})}{V_{lip}} \tag{S2}$$

Here, V_{in} and V_{out} are the volumes of the inner (1 cm^{-3}) and outer solutions (5 cm^{-3}) in the dialysis tube, respectively. V_{lip} is the volume of the BLM phase of all liposomes, which was calculated from PC concentration [PC], determined using an *in vitro* assay kit; the thickness of the BLM (x = 5 nm) and the molecular area of the PC (A = 0.456 nm^2/molecule[5,6]) according to Eq. (S3).

$$V_{lip} = [PC]V_{out}N_A Ax/2 \tag{S3}$$

where N_A is the Avogadro constant. In the calculation of R, the concentration of $R6G^+$ in the internal aqueous phase of the liposome was assumed to be $[R6G^+]_W$. Even if the amount of $R6G^+$ transferring

into the internal aqueous phase of the liposome was low, the effect on *R* was considered negligible because the volume of the internal aqueous phase was about 1% of the total volume of the outer and inner solutions.

The measured $[R6G^+]_{mea}$ and $[R6G^+]_{ref}$ are shown in Fig. S1.

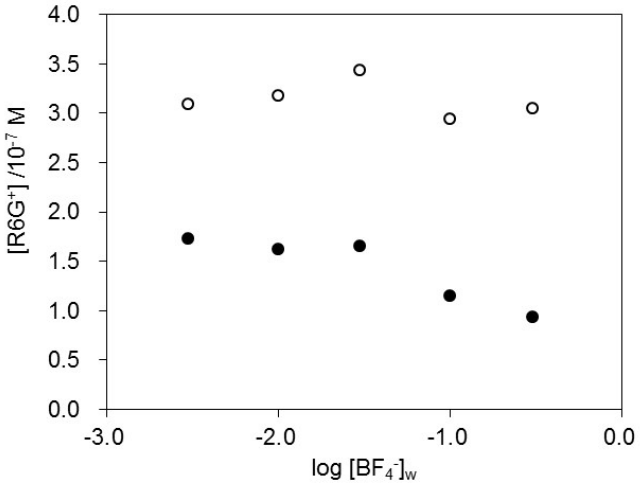

Fig. S1 Dependence of $[R6G^+]_{ref}$ and $[R6G^+]_{mea}$ in the presence of PC (●, $[R6G^+]_{mea}$) and in the absence of PC (○, $[R6G^+]_{ref}$) upon the concentration of BF_4^-. Original composition of the aqueous solution: 1.0×10^{-1} mol dm^{-3} phosphate buffer (pH 7.0), 1.8×10^{-7} mol dm^{-3} R6GCl, and x mol dm^{-3} $NaBF_4$ (x = 3.0×10^{-3}, 1.0×10^{-2}, 3.0×10^{-2}, 1.0×10^{-1} or 3.0×10^{-1}). PC and cholesterol concentration add as liposome: 3.3×10^{-3} mol dm^{-3}.

Adsorption of $R6G^+$ on the liposome surface with the PC:CH ratio of 1:1

The total mole number per unit area of adsorption sites, N_{ads}^T on the liposome with the PC:CH ratio of 1:1 was evaluated by the liposome extraction described above, which is same procedure in

previous paper[4]. The liposome extraction was performed in changing the $R6G^+$ concentration in aqueous phase from 2.5×10^{-4} mol dm^{-3} to 1.0×10^{-3} mol dm^{-3}. The $R6G^+$ was added as a chloride salt. In this condition, R was independent upon concentration of Cl^-; $R6G^+$ and Cl^- are undistributed into the liposome membrane. It was assumed that the decrease in $[R6G^+]_W$ was caused by the adsorption of $R6G^+$ on the liposome surface. The mole number of $R6G^+$ adsorbed on the liposome surface, $N_{R6G+,ads}$, was plotted to $[R6G^+]_W$ (Fig. S2). $N_{R6G+,ads}$ increased with the increase of $[R6G^+]_W$ and reached the saturated adsorption at 1.0×10^{-3} mol m^{-2}. Therefore, N_{ads}^T was assumed to be 0.85 $\times 10^{-7}$ mol m^{-2}.

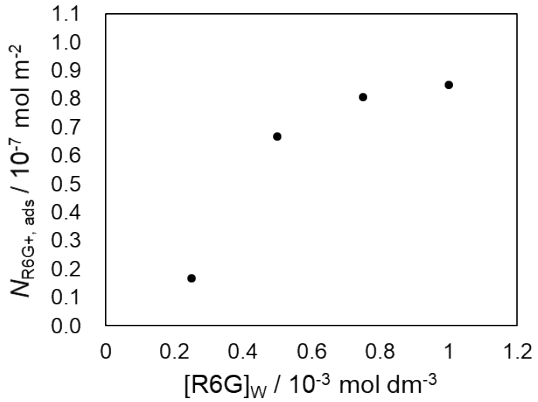

Figure S2. (a) Equilibrium isotherm for the adsorption of $R6G^+$ on the surface of a liposome. Original composition of the aqueous solution: x mol dm^{-3} $R6G^+$ (2.5×10^{-4}, 5.0×10^{-4}, 7.5×10^{-4} or 1×10^{-3}), 1.0×10^{-1} mol dm^{-3} phosphate buffer (pH 7) and 1.0×10^{-3} mol dm^{-3} NaCl. PC and cholesterol concentration add as liposome: 3.3×10^{-3} mol dm^{-3}.

Evaluation of K_D, K_{ip} and K_{ads} by analysing R

The R values were calculated from the experimental results of Fig. S1 according to Eqs. (S1–S3). R is expressed by Eq. (S4)[4].

$$R = K_{ip}K_D\left[BF_4^-\right]_W + \sqrt{\frac{K_D}{\left[R6G^+\right]_W}}\sqrt{\left[BF_4^-\right]_W} + \frac{2N_{ads}^{T}K_{ad}}{x\left(1 + K_{ad}\left[R6G^+\right]_W\right)} \quad \text{(S4)}$$

The obtained R was plotted against $([BF_4^-]_W)^{1/2}$, as shown in Fig. S3 and analyzed using Eq. (S4) by quadratic curve approximation.

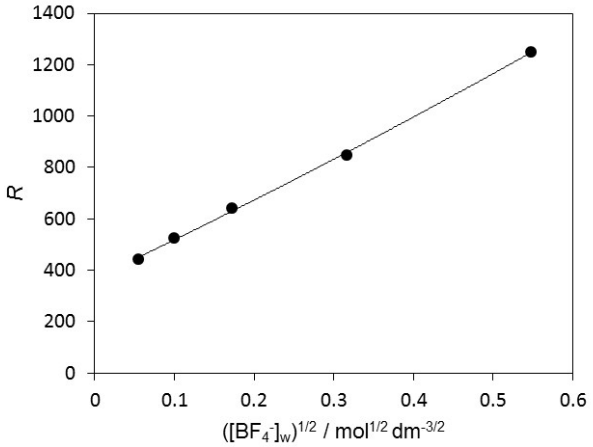

Fig. S3 Dependence of R estimated from ($[R6G^+]_{ref}$ – $[R6G^+]_{mea}$) in Fig. S1 upon the concentration of the BF_4^-. The solid line indicates an approximate curve analyzed according to Eq. (S4).

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